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Research Article

CHEMICAL COMPOSITION AND ANTIBACTERIAL ACTIVITY OF THE VOLATILE OIL FROM THE BARK OF *GLIRICIDIA SEPIUM*

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ABSTRACT

Nineteen compounds have been identified and quantified from the bark oil of *Gliricidia sepium* by GC-MS analysis. The major components are methyl-3(E)-pentenyl ether (11.55%), 3-methyl-2-butanol (10.65%), 3-methoxy hexane (10.14%), 1-(1-ethoxyethoxy)-2-hexene (9.72%), 2-decanol (8.97%), coumarin (8.07%) and hexadecanoic acid (5.16%). The antibacterial activity of the essential oil was checked against various pathogenic bacteria such as *Bacillus cereus, Enterobacter faecalis, Salmonella paratyphi, Staphylococcus aureus, Escherichia coli, Streptococcus faecalis, Proteus vulgaris, Klebsiella pneumoniae, Pseudomonas aeruginosa* and *Serratia marcescens* by 'agar well diffusion' method. The bark oil exhibited pronounced activity against all tested microorganisms and its activity is quite comparable with the standard antibiotics screened under similar conditions. The remarkable antibacterial activity exhibited by the bark oil can be attributed to the long chain fatty acids and alcohols present in it. This study shows that the *Gliricidia sepium* bark essential oil can be used as a potential external antiseptic and can be incorporated into the drug formulations.

Keywords: *Gliricidia sepium*, essential oil, antibacterial activity, agar well diffusion method, standard antibiotics, drug formulation.

INTRODUCTION

Gliricidia sepium (Leguminosae family) is a medium sized tree introduced into India from the American continent. This tree is used in Mexico as shade for cocoa and coffee plantations and for this reason it is called 'Madrecacao' (mother of cocoa). It is also used as a poison for rodents and in fact the Latin name *Gliricidia* means rodent poison. It is used as a hedge plant and the flowers are utilized as food in some places in Mexico[1]. In Panama, decoction of leaves used in utricaria, rash and also in burns and erysepalas[2]. In Guatemala and Costa Rica, bark decoction is used against bacterial and protozoal infections[3]. Branches of *Gliricidia sepium* is used to reduce fever in children and adults. It has also been used to treat infections produced by *Microsporum canis*, *Trychophyton mentagrophytes* and *Neisseria gonorrohae*[4]. Sharma and Qadry investigated the larvicidal activity of the crude ethanol extract of *Gliricidia sepium* bark and leaves[5]. Various phytochemicals like flavanoids[6], triterpenoid saponins[7], stigmastanol glucoside [8], rhamnogalactoside of kaempferol[9], coumarin, coumaric acid and melilotic acid[10] have been isolated and characterised from various parts of this plant. Allelochemicals from *Gliricidia sepium* leaves were extracted, identified and quantified using HPLC[11]. Rastrelli isolated a new 12a-hydroxy rotenoids from the methanolic extract of *Gliricidia sepium* bark[12].

Microbiological assays are used for the quantitative determination of antibiotics and inhibitory chemical agents and also the determination of the sensitivity of the microorganisms to these agents. Synthetic chemicals have their side effects and the development of bacterial resistance to the presently available antibiotics has necessitated the search for new antimicrobial agents. So we look into the nature as an ally and resource in finding new strategies to combat diseases of plants, animals and human beings.

Most of the previous chemical investigations on *Gliricidia sepium* have focused mainly on the isolation of potential allelopathic and toxic compounds from heart wood, leaves and roots of the plant. So far no data about the volatiles from G. *sepium* bark and its antibacterial activity have been published. In this work, the antibacterial property of the *G. sepium* bark oil was checked against ten pathogenic bacteria namely *Bacillus cereus, Enterobacter faecalis, Salmonella paratyphi, Staphylococcus aureus, Escherichia coli, Streptococcus faecalis, Proteus vulgaris, Klebsiella pneumoniae, Pseudomonas aeruginosa* and *Serratia marcescens*. In addition, chemical composition of the volatile compounds of the bark oil, were also determined.

MATERIALS AND METHODS

Plant material

Gliricidia sepium bark was collected from Kerala, South India and authenticated by Dr. A.K. Pradeep, Dept. of Botany, Calicut University. Voucher specimen is deposited in the specially maintained herbarium, Department of Chemistry, Calicut University.

Essential oil extraction

The fresh bark (250g) of *Gliricidia sepium* was cut into small pieces and ground to a paste using an electric mixer grinder and subjected to steam distillation for three hours. About 2 liters of the distillate were collected and extracted with diethyl ether (3X100 ml) and dried using anhydrous sodium sulphate. The dry ether extract on evaporation yielded 0.39g (0.15% of fresh weight of the sample) of pale yellow oil. Chemical composition of the bark essential oil was analyzed by GC-MS (table 1).

Gas chromatography – Mass spectrometry

The GC-MS analyses were carried out on Agilent 6890 GC system equipped with a 5973 inert mass selective detector (Agilent Technologies, USA). A CO Sil 8 CB (Varian, Middleburg, Netherlands) column of 30m length, 0.25mm i.d, and 0.25µm film thickness was used. The oven was programmed from an initial temperature 50^{0}C (hold for 2 min) to the final temperature 280^{0}C at the rate of 10^{0}C/min. The final temperature hold up time was 5min. Helium at the rate of 1 ml/min was used as the carrier gas in constant flow mode. The inlet and interface temperatures were kept at 280^{0}C. The EI source was operated at 230^{0}C and the quadrupole temperature was 150^{0}C. The MS was scanned from 30 to 500 mass units. One micro litre of the sample was injected in split mode at a split ratio of 10:1. For compound identifications, Wiley 275 library spectra were used (online).

Test microorganisms

The microorganisms used for antibacterial activity evaluation were obtained from Microbial Type Culture Collection and gene bank (IMTECH, Chandigarh, India). They were *Bacillus cereus* (MTCC-1305), *Enterobacter faecalis* (MTCC-5112), *Salmonella paratyphi,* (MTCC-735), *Staphylococcus aureus* (MTCC-96), *Escherichia coli* (MTCC-729), *Streptococcus faecalis* (MTCC-439), *Proteus vulgaris* (MTCC-426), *Klebsiella pneumoniae* (MTCC-109), *Pseudomonas aeruginosa* (MTCC-647) and *Serratia marcescens* (MTCC-86).

Culture medium and inoculum

The stock cultures of microorganisms used in this study were maintained on Plate Count Agar slants at 4^0C. Inoculum was prepared by suspending a loop full of bacterial cultures into 10ml of nutrient broth and was incubated at 37^0C for 24hours. On the next day Muller-Hinton agar (MHA) (Merck) sterilized in a flask and cooled to 45-50^0C was distributed by pipette (20ml) into each sterile Petri dish and swirled to distribute the medium homogeneously. About 0.1ml of bacterial suspension was taken and poured into Petri plates containing 20ml nutrient agar medium. Using the L-shaped sterile glass spreader bacterial suspensions were spread to get a uniform lawn culture.

Antibacterial activity assay

The agar diffusion method is used for the antimicrobial evaluations. Wells of 8mm (0.8cm) diameter were dug on the inoculated nutrient agar medium with sterile cork borer and 50 µl of the bark essential oil (at various concentrations) were added in each well. The essential oils of required concentrations 60%, 15%, 10%, 5% and 1% were prepared by dissolving the oils into appropriate quantities of DMSO, which did not influence the growth of bacteria was used as a negative control. The plates were then incubated at 37^oC overnight and examined for zone of inhibition. The diameter of the inhibition zone was measured in mm. The standard antibiotic drugs tobramycin, gentamicin sulphate, ofloxacin and ciprofloxacin were also screened under similar conditions for comparison. An extract was classified as active when the diameter of the inhibition was equal to or larger than 8 mm[13]. All the assays were performed in triplicate and expressed as average values.

RESULTS AND DISCUSSION

Nineteen known compounds have been identified and quantified from the bark essential oil of *Gliricidia sepium* by GC-MS analysis (table 1). The major compounds present in the bark essential oil are methyl-3(E)-pentenyl ether (11.55%), 3-methyl-2-butanol (10.65%), 3-methoxy hexane (10.14%), 1-(1-ethoxyethoxy)-2-hexene (9.72%), 2-decanol (8.97%), coumarin (8.07%) and hexadecanoic acid (5.16%).

Gliricidia sepium bark oil at various concentrations were evaluated for antimicrobial activity against Gram-positive and Gram-negative bacteria strains and the oil exhibited marked activity against all tested microorganisms. As can be seen from table 2, the bark oil (60%) showed pronounced activity against *Bacillus cereus, Enterobacter faecalis, Salmonella paratyphi, Escherichia coli, Proteus vulgaris*, *Pseudomonas aeruginosa, Serratia marcescens, Staphylococcus aureus, Streptococcus faecalis* and *Klebsiella pneumonia* (42-64mm/50µl inhibition zone). The activities of 15%, 10%, 5% and 1% oil samples were also studied against various pathogenic bacteria and the results obtained were compared with standard antibiotics. The *G. sepium* bark oil at a concentration of 15% was found to be active against all the microorganisms tested (16-26mm/50µl inhibition zone). 10% and 5% oil samples also performed appreciable inhibitory activity on all microorganisms (13-21mm/50µl inhibition zone) and (11-18mm/50µl inhibition zone) respectively. The activity of 1% oil was rather less, as the activity is dose dependent, yet it showed pronounced activity on *Streptococcus faecalis* and *Escherichia coli* (14-15mm/50µl inhibition zone).

Table 1: Composition of the bark essential oil of *Gliricidia sepium*

Identified compounds	Percentage
1-hexanol	1.92
1-(1-ethoxyethoxy)-2-hexene	9.72
2-ethyl hexanol	2.61
3-methyl-2-butanol	10.65
3-methoxy hexane	10.14
2-decanol	8.97
methyl-3(E)-pentenyl ether	11.55
2,4-dimethyl-3-hexanol	2.79
methyl-3(E)-hexenyl ether	2.19
methyl-3-butenyl ether	1.02
coumarin	8.07
4-ethoxy ethyl benzoate	2.1
caryophyllene oxide	3.05
dodecanoic acid	2.37
humulene epoxide	3.64
T-muurolol	1.77
octadecanal	1.11
tetradecanoic acid	1.23
hexadecanoic acid	5.16

Table 2: Antibacterial activity of the bark oil of *Gliricidia sepium*

Microorganisms	Diameter of inhibition zones(mm/50µl)				
	***G. sepium* bark oil**				
	60 %	**15 %**	**10 %**	**5 %**	**1 %**
Bacillus cereus	64	18	15	13	11
Enterobacter faecalis	42	17	13	11	10
Salmonella paratyphi	42	18	14	12	11
Staphylococcus aureus	52	22	16	14	12
Escherichia coli	52	24	19	16	14
Streptococcus faecalis	52	26	21	18	15
Proteus vulgaris	40	16	14	12	11
Klebsiella pneumoniae	50	25	18	16	13
Pseudomonas aeruginosa	52	18	15	13	11
Serratia marcescens	52	26	20	16	13

Used Concentrations: 50µl of 60%, 15%, 10%, 5% and 1% of the bark essential oil in DMSO

Table 3: Inhibition zones formed by the standard antibiotics—tobramycin, gentamicin sulphate, ofloxacin, ciprofloxacin and negative control

Microorganisms	Diameter of inhibition zones(mm/50µl)				
	Tob 10 µg	Gen 10 µg	Oflo 10 µg	Cip 10 µg	Control (DMSO)
Bacillus cereus	28	32	34	30	-
Enterobacter faecalis	26	32	32	26	-
Salmonella paratyphi	25	30	28	30	-
Staphylococcus aureus	26	28	24	24	-
Escherichia coli	30	36	32	34	-
Streptococcus faecalis	28	34	30	32	-
Proteus vulgaris	26	30	24	32	-
Klebsiella pneumoniae	26	32	32	36	-
Pseudomonas aeruginosa	26	24	32	28	-
Serratia marcescens	24	32	30	30	-

Tob: tobramycin, Gen: gentamicin sulphate, Oflo: ofloxacin, Cip: ciprofloxacin

The antibacterial spectra of the *G. sepium* bark essential oil showing zone of inhibition in millimetres, for Gram-positive and Gram-negative bacteria are shown in table 2. In addition, the inhibition zones formed by standard antibiotics and those of negative control are listed in table 3.

It was observed that the inhibitory effect of 15% *G. sepium* bark oil (contains 3µg of fatty acids and alcohols) on *Staphylococcus aureus, Escherichia coli, Streptococcus faecalis, Klebsiella pneumoniae* and *Serratia marcescens* was comparable with that of the standard tobramycin at a concentration of 10µg. So it can be used as an external antiseptic in prevention and treatment of bacterial infections. The long chain fatty acids and alcohols are reported to have antimicrobial activity[14,15]. The remarkable antibacterial activity exhibited by the bark oil of G. *sepium* can be attributed to the presence of these long chain fatty acids and alcohols.

CONCLUSION

From the above experiment it can be inferred that *Gliricidia sepium* bark essential oil showed significant activity against Gram-positive and Gram-negative bacteria. The activity of bark oil was found to be quite comparable with the standard antibiotics screened under similar conditions. So the oil can be used as an external antiseptic in the prevention and treatment of bacterial infections caused by various pathogenic bacteria such as *Bacillus cereus, Enterobacter faecalis, Salmonella paratyphi, Staphylococcus aureus, Escherichia coli, Streptococcus faecalis, Proteus vulgaris, Klebsiella pneumoniae, Pseudomonas aeruginosa* and *Serratia marcescens*, which have developed resistance to antibiotics. The incorporation of this oil into the drug formulations is also recommended.

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